

to the native membrane environment. To improve the solubilization of membrane proteins (MPs) and allow their study in bicellar systems, D6PC was replaced by detergents from the monoalkylphosphocholine (MAPCHO) family of which dodecylphosphocholine (DPC) is known for its ability to solubilize MPs. More specifically DPC, tetradecyl- (TPC) and hexadecylPC (HPC) have been employed. To verify the possibility of making bicelles with different hydrophobic thickness to better accommodate MPs, D14PC was also replaced by phospholipids with different acyl chain lengths: dilauroylPC (D12PC), dipalmitoylPC (D16PC) and distearoylPC (D18PC). Preliminary results obtained by <sup>31</sup>P solid-state NMR at several lipid-to-detergent molar ratios (q) and temperatures indicate that magnetically-oriented bicelles can be formed with D12PC/DPC (q=2, 17–47°C), D14PC/DPC (q=2–3, 32–52°C), D16PC/DPC (q=1.6–2.4, 42–47°C), D14PC/TPC (q=2, 32–57°C) and D16PC/TPC (q=1.6–2.4, 42–52°C). The temperature range at which these bicelles orient is, thus, dictated by the gel-to-fluid phase transition temperature of the phospholipids. Moreover, the longer the phospholipid chain length, the smaller the q ratio range at which bicelles orient. These results will be discussed in terms of PCs and MAPCHOs solubility. These are promising model membranes that could be amenable to both solution- and solid-state NMR, thus enabling structure determination with different bilayer thickness as well as the study of lipid interactions with a single membrane mimicking system.

#### 2597-Pos Board B289

##### Fluorescence Measurements of Aromatic Amino Acids in the Presence of Lipid Membranes

**Sirine Khelifi**, Merrell A. Johnson, Bruce D. Ray, Horia I. Petrache. Physics, IUPUI, Indianapolis, IN, USA.

Amphiphilic peptides are capable of finding their way to, and occasionally through, cellular membranes using a mechanism that includes specific amino acid sequences. Physical measurements of amino acid-lipid interactions are of interest for a quantitative description of peptide affinities to biological membranes. In this study, we investigate small peptide-lipid interactions using the fluorescence of the aromatic amino acids tyrosine (Tyr), tryptophan (Trp) and phenylalanine (Phe). Reference spectra in isopropanol-water, ethylene-glycol-water, DMSO-water, and polyethyleneglycol-water solutions are obtained to mimic hydrophobic environments and are used to quantify the interaction of Lys-Tyr-Lys, Trp-Gly, and Gly-Phe with 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS) lipid membranes. These fluorescence data complement previously reported UV absorption data and have the advantage of eliminating background and scatter from solution. Together with NMR data, these results can be used to more fully characterize lipid-aromatic amino residue interactions.

#### 2598-Pos Board B290

##### Single Molecule Studies of PKC $\alpha$ Activation Mechanism on Membrane Surfaces

**Brian P. Ziemba**, Joseph J. Falke.

Biochemistry, University of Colorado, Boulder, CO, USA.

The master kinase PKC $\alpha$  is a central player in the normal function of many signaling pathways, and also plays a role in multiple pathologies. The conventional model for activation of classical PKCs, including PKC $\alpha$ , proposes two major steps during conversion of inactive, cytoplasmic enzyme to active, membrane-bound enzyme. First, a cytoplasmic Ca<sup>2+</sup> signal activates C2 domain that serves to recruit the inactive enzyme from cytoplasm to the PS-rich inner leaflet of plasma membrane. Second, inactive enzyme, bound to plasma membrane via its C2 domain, is activated by appearance of the second messenger lipid diacylglycerol (DAG) that recruits the inhibitory C1A and C1B domains from the kinase domain to the membrane, thus activating the kinase. To test the predictions of this model, we have employed single molecule methods to analyze membrane binding and surface diffusion of full length PKC $\alpha$ , and also simpler constructs containing a subset of its domains. TIRF microscopy was used to visualize these proteins on supported lipid bilayers containing different combinations of target lipids in order to investigate the contributions of individual domains to membrane binding and lipid-induced kinase activation. Previous studies have shown that single molecule TIRF analysis of peripheral proteins on supported lipid bilayers provides extensive information on protein-lipid interactions difficult to obtain by other methods (Ziemba, Knight & Falke (2012) *Biochemistry* 51(8):1638–1647. Knight, Lerner, Marciano-Velazquez, Pastor & Falke (2010) *Biophys J* 99:2879–87). The present single molecule analysis reveals a previously unknown intermediate in the PKC $\alpha$  activation reaction, and indicates this is the major intermediate while the enzyme awaits the appearance of activating diacylglycerol or phorbol ester. The findings yield new insights into the

PKC $\alpha$  activation mechanism, and show that the single molecule approach provides a new window into the activation mechanism of membrane-bound signaling enzymes.

#### 2599-Pos Board B291

##### The Ebola Virus Matrix Protein Bends Biological Membranes

**Smita P. Soni**<sup>1</sup>, Robert V. Stahelin<sup>1,2</sup>.

<sup>1</sup>Biochemistry & Molecular Biology, IU- School of Medicine, South Bend, IN, USA, <sup>2</sup>Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN, USA.

Ebola is one of the most virulent pathogen that causes severe hemorrhagic fever with fatality rate as high as 90%. There is an urgency in the development of treatment as there are no known drugs or vaccines approved by the FDA and the virus poses a serious health and potential biological threat. The Ebola viral matrix protein 40 (VP40), is the most abundantly expressed protein of the virus and alone harbors the ability to form virus like particles (VLPs) that are indistinguishable from the authentic virus. The mechanism of VP40 assembly at the plasma membrane before the release of the virions remains poorly understood. In order to better understand the process of viral egress, it is crucial to understand how VP40 is able to bend the plasma membrane to regulate formation of VLPs. Here we take a detailed look at how VP40 alone is able to bend giant unilamellar vesicles (GUVs) membranes, a model for VLP egress. Here, we imaged GUV's containing PC:PE with and without anionic lipids enriched in the plasma membrane such as PS and phosphoinositides to determine VP40 budding selectivity. The results demonstrate PS-dependent bending and vesiculation from GUV's membrane. The vesiculation is not enhanced in the presence of cholesterol and completely inhibited in GUVs composed of polyvalent phosphoinositides. In concert with budding from live human cell models, the bending and VLP formation is unique to PS containing membranes. GUVs have previously been utilized to understand membrane scission and to expose the role of viral proteins in membrane bending. Elucidating the effect of VP40 on GUVs composed of lipids that mimic biological membranes will help understand the mechanistic details of an otherwise illusive membrane remodeler.

#### 2600-Pos Board B292

##### Palmitoylation as a Key Factor to Understand Sp-C-Lipid Interactions in the Lung Surfactant System

**Nuria Roldan**<sup>1</sup>, Erik Goormaghtigh<sup>2</sup>, Jesus Perez-Gil<sup>1</sup>, Begoña Garcia-Alvarez<sup>1</sup>.

<sup>1</sup>Universidad Complutense Madrid, Madrid, Spain, <sup>2</sup>Universite Libre de Bruxelles, Brussels, Belgium.

Surfactant protein C (SP-C) has been regarded as the most specific protein linked to lung development. So far, great efforts have been done to understand the structure-function relationships of this lipopeptide, nevertheless its high hydrophobicity and tendency to aggregate forming amyloid-like structures have made its study a challenging task. Previous evidence has pointed out the importance of SP-C palmitoylation in sustaining the proper dynamics of lung surfactant, but the mechanism by which this posttranslational modification stabilizes the interfacial surfactant film under dynamic compression-expansion cycles mimicking the process of breathing, is still unrevealed. In this work we have compared the behavior of a native palmitoylated SP-C with a non-palmitoylated recombinant SP-C (rSP-C) in membrane environments by means of ATR-FTIR spectroscopy. Our results suggest that palmitoylation modulates SP-C-lipid interactions and besides, it may play a dual role in combination with electrostatic interactions with anionic phospholipids (POPG) to maintain a proper SP-C conformation in the lung surfactant context. Functional approaches will provide further insights into SP-C palmitoylation-induced effects, allowing the characterization of SP-C structure-function determinants.

#### 2601-Pos Board B293

##### Clarifying the Roles of Cardiolipin

**Sanja Pöyry**<sup>1</sup>, Oana Cramariuc<sup>1</sup>, Pekka Postila<sup>1</sup>, Karol Kaszuba<sup>1</sup>, Marcin Sarewicz<sup>2</sup>, Artur Osyczka<sup>2</sup>, Tomasz Rog<sup>1</sup>, Ilpo Vattulainen<sup>1</sup>.

<sup>1</sup>Dept. of Physics, Tampere Univ Technology, Tampere, Finland,

<sup>2</sup>Jagiellonian Univ, Krakow, Poland.

Cardiolipins (CL) are uniquely structured double lipids that are universally found in membranes which couple electron transport and phosphorylation. The proposed roles of CL in these membranes include mainly two aspects: the effects on the structure and dynamics of the membranes' lipid component and the interplay with membrane-associated proteins. In previous work, we have studied both of these aspects by utilizing atomistic molecular dynamics simulations, starting with the general effects of CL on membrane properties and the interplay with ions [1], further moving on to the interactions with the